

P A T E N T   C L A I M S

Sub E3  
 1. A modified human TNF $\alpha$  molecule capable of raising neutralizing antibodies towards wild-type human TNF $\alpha$  following administration of said modified TNF $\alpha$  molecule to a human host, wherein at least one peptide fragment of the human TNF $\alpha$  molecule has been substituted by at least one peptide known to contain an immunodominant T cell epitope or a truncated form of said molecule containing an immunodominant <sup>T cell</sup> epitope and one or both flanking regions of the human TNF $\alpha$  molecule comprising at least one TNF $\alpha$  B cell epitope, wherein the substitution introduces a substantial change in the amino acid sequence of any one of the strands of the front  $\beta$ -sheet, in any one of the connecting loops and/or in any one of the B', I or D strands of the back  $\beta$ -sheet.

Modified  
 2. ~~A modified human TNF $\alpha$  molecule capable of raising neutralizing antibodies towards wild-type human TNF $\alpha$  following administration of said modified TNF $\alpha$  molecule to a human host, wherein at least one peptide fragment of the human TNF $\alpha$  molecule has been substituted by at least one peptide known to contain an immunodominant T cell epitope or a truncated form of said molecule containing an immunodominant epitope and one or both flanking regions of the human TNF $\alpha$  molecule comprising at least one TNF $\alpha$  B cell epitope, wherein said modified TNF $\alpha$  molecule is substantially free from TNF $\alpha$  activity.~~  
 according to claim 1

Modified  
 3. ~~A modified human TNF $\alpha$  molecule according to claim 2, wherein the modified TNF $\alpha$  molecule, when tested in the L929 bioassay, is substantially free from TNF $\alpha$  activity, and wherein antibodies raised against the modified TNF $\alpha$  molecule in a suitable host significantly inhibit the activity of native TNF $\alpha$  in the L929 bioassay, and/or wherein said antibodies significantly inhibit the binding~~

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Sub 4  
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8. Modified human TNF $\alpha$  molecule according to ~~claims 1-4~~,  
wherein the substitution comprises segments of the H and  
I strands and the entire connecting loop, preferably  
amino acids 132 to 152

9. Modified human TNF $\alpha$  molecule according to ~~claims 1-4~~,<sup>claim 1</sup> wherein the substitution comprises a segment of the D strand, at least a segment of the E strand and the entire connecting loop, preferably amino acids 65 to 79 or 64 to 84.

10. Modified human TNF $\alpha$  molecule according to ~~claims 1-4~~,<sup>claim 1</sup> wherein the substitution comprises the entire C' and C strands and a segment of the D strand, preferably amino acids 40 to 60.

11. Modified human TNF $\alpha$  molecule according to ~~claims 1-4~~,<sup>claim 1</sup> wherein the substitution comprises at least a segment of the E strand and of the front  $\beta$ -sheet of one or both of the connecting loops, preferably amino acids 76 to 90.

12. Modified TNF $\alpha$  according to ~~claims 1-4~~,<sup>claim 1</sup> having the amino acid sequence shown in SEQ ID NO:8.

13. Modified TNF $\alpha$  according to ~~claims 1-4~~,<sup>claim 1</sup> having the amino acid sequence shown in SEQ ID NO:10.

14. Modified TNF $\alpha$  molecule according to ~~claims 1-4~~,<sup>claim 1</sup> having the amino acid sequence shown in SEQ ID NO:4 or SEQ ID NO:16.

15. Modified TNF $\alpha$  according to ~~claims 1-4~~,<sup>claim 1</sup> having the amino acid sequence shown in SEQ ID NO:20.

16. Modified TNF $\alpha$  according to ~~claims 1-4~~,<sup>claim 1</sup> having the amino acid sequence shown in SEQ ID NO:14.

17. Modified human TNF $\alpha$  molecule according to ~~any of claims 1-11~~,<sup>claim 1</sup> wherein the inserted T cell epitope is promiscuous and known to be immunogenic in a majority of human HLA class II types.

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Claims

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to claim  
quence.  
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claim 1  
~~any of~~

~~claim~~

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tically acceptable adjuvant, ~~such as aluminium phosphate, aluminium hydroxide, calcium phosphate, muramyl dipeptide or iscom.~~

28. A vaccine according to claim 27 for the prevention or treatment of <sup>disease</sup> ~~diseases~~ promoted by TNF $\alpha$  release or activity ~~such as chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases, including Crohn's disease and Colitis Ulcerosa, and cancer, disseminated sclerosis, diabetes, psoriasis, osteoporosis and asthma.~~

29. A vaccine against TNF $\alpha$  comprising isolated DNA which codes for the modified human TNF $\alpha$  molecule according to ~~any one of claims 1-18~~ <sup>claim 1</sup> inserted in a suitable expression vector.

30. A vaccine according to claim 29 containing a construct comprising a non-infectious non-integrating DNA sequence encoding a modified TNF $\alpha$  molecule ~~according to any of claims 1-18~~ operatively linked to a promoter sequence which can control the expression of said DNA sequence in humans, in an amount sufficient that uptake of said construct occurs, and sufficient expression occurs to induce a neutralizing antibody response against TNF $\alpha$ .

31. A vaccine according to claim 29, comprising a viral expression vector, ~~such as a retroviral expression vector.~~

32. A vaccine according to <sup>claim 27</sup> ~~any one of claims 27-31~~ for oral or parenteral, ~~e.g. subcutaneous, intramuscular or intradermal~~ administration.

33. The use of antibodies raised by administering a vaccine according to <sup>claim 27</sup> ~~any one of claims 27-32~~, preferably monoclonal antibodies.

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36. A diagnostic method for TNF $\alpha$ -related diseases employing an in vitro immunoassay to detect TNF $\alpha$  in human body fluids.

39. Use of a modified TNF $\alpha$  molecule for the preparation of a medicament for the treatment or prevention of diseases the pathophysiology of which is at least partially due to TNF $\alpha$  release or activity.

add #1

SUB  
D2